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(54) Title: SOURCE OF NUCLEI FOR NUCLEAR TRANSFER

(57) Abstract

The reconstruction of a mammalian embryo uses lymphocytes as the source of donor nuclei. The recipient may be an enucleated oocyte. The embryo so prepared may be brought to term, used in recloning techniques or used to prepare embryonic stem cell lines.

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Source of nuclei for nuclear transfer

This invention relates to the generation of animals genetically identical to an existing or existed animal. Further, during the process of regeneration some characteristic(s) can be changed by recombinant DNA technology to produce a transgenic animal by the addition or deletion of selected genes.

Known procedures for nuclear transfer involve the transfer of a nucleus taken from a pre-implantation stage embryo into an enucleated mature oocyte. Following activation of the oocyte, in a process that mimics sperm entry and signalling, an embryo develops and eventually an individual that is genetically (as far as DNA is concerned) identical to the donor embryo. The limited number of cells present in a mammalian pre-implantation embryo, however, allows the regeneration of a limited number of embryos. Pre-implantation embryo nuclei donors do not allow the use of recombinant DNA technology because of the limited number of cells available. Most importantly, though, the genetic value of the embryo, and thus of the animal that will be born, can only be estimated. This is of low economic value. For these reasons the potential of nucleus transfer technology has not been developed with commercial exploitation in mind; its major use is for scientific purposes.

A partial solution to the limited number of nuclei has been the use of a so called 'serial nucleus transfer' where the embryos obtained from the starting embryos are further subjected once, or more than once, to the same procedure therefore increasing the number of embryos regenerated (Stice & al., 1991, Theriogenology 35, 273).

The major limitations to the use of nucleus transfer procedure outlined above would be overcome if a renewable and / or unlimited source of nuclei to be used in the process could be made available. For many years people have attempted to establish cell lines from pre-implantation embryos (embryonic stem cell lines) but failed except for the mouse. Such work is reviewed in Galli et al. 1994, Zygote 2: 385-389. This type of cell would represent the ideal source of nuclei,

however in the mouse they have never been used in nucleus transfer experiments.

Cultured inner cell mass cells or presumptive embryonic cell lines have been obtained and successfully used for nucleus transfer experiments to produce embryos: Moor, Sun & Galli, 1992, Animal Reprod. Sci. 28, 423-431; Stice & al. 1996, Biol. Reprod. 54, 100-110. Viable offspring have been produced in cattle and sheep: Sims & First, 1994, Proc. Natl. Acad. Sci. USA 91: 6143-6147; Campbell & al. 1996, Nature 380, 64-66; Wells & al. 1997, Biol. Reprod. 57,385-393. More recently, viable offspring has also been obtained with the use of nuclei from cultured fetal cells: Wilmut & at. 1997, Nature 385, 810-813; see The New York Times 21 January 1998 and Nature 392, 113, 1998. One lamb has been produced from a sample taken from a primary culture containing mainly mammary epithelial cells of an adult sheep: Wilmut & at. 1997, Nature 385, 810-813.

The advantages of using a renewable source of cell or a cell line in nucleus transfer procedure are:

- cells can be easily collected and cultivated or possibly stored in liquid nitrogen;
- an unlimited number of embryos could be produced over a long period;
- cells can readily be modified in vitro using recombinant DNA technology.

There has been discussion about using nuclei of somatic cells collected from adult animals. This will have particular application for livestock species where the value of an animal is determined by his progeny if a sire or by her production records if, for example, a dam. To regenerate a unique animal for production or genetic characteristics (transgenic) it is imperative to use nuclei from an animal which is an adult or one which has at least been born alive. That is not the case for the work using fetal or embryonic cells as a source of nuclei.

Description of the invention

The present invention provides a method of reconstructing a mammalian embryo, the method comprising transferring a lymphocyte into a suitable recipient.

The lymphocyte can be transferred intact, optionally with a broken cell membrane, or the nucleus may be extracted and used for transfer. Preferably, the lymphocyte is transferred with the cell membrane broken.

Preferably, this invention finds application in the reconstruction of embryos of mammals using donor cells and recipients from the same species, preferably to reconstruct ungulate species embryos. The lymphocyte may be collected from an adult animal or an animal from a viable birth.

The invention further provides a method of reconstructing a mammalian embryo comprising reconstructing a first generation embryo by the steps of a method according to the first aspect of the invention and then transferring a cell from the said first generation embryo to a suitable recipient to form a second generation embryo.

The invention still further provides a method of preparing a mammal, the method comprising reconstructing a mammalian embryo using a method described above; allowing the embryo so produced to develop to term; and, optionally, breeding from the animal so formed.

The present invention further provides a method of preparing embryonic stem cell lines, comprising reconstructing an animal, preferably mammalian embryo using a method described above; and transferring the embryo to a culture system.

The present invention further provides a method of preparing embryonic stem cell lines, comprising reconstructing an animal, preferably mammalian embryo using a method described above; isolating the inner cell mass of the embryo from the embryo; and transferring the inner cell mass to a culture system.

The culture system allows the embryo cells to attach, outgrow and produce a cell line with embryonic characteristics. The term "embryo" used herein includes morulas (8-16 cells), morulas (16-32 cells) and blastocysts (64 cells and above). The embryo has a reasonable (about 50% or more) chance of development to an established pregnancy.

The present invention utilises lymphocytes and their derivatives or precursors. The donor cell, whilst usually a terminally differentiated hematopoietic cell, could be at a partially differentiated stage. These are mononuclear cells of hematopoietic lineage, present in bone marrow, lymphoid organs and in peripheral blood. They are also found in the umbilical cord of the new-born. The intact cells, cells with their membranes broken prior to transfer or the isolated nuclei are used as a source of nuclei in conventional nucleus transfer procedures. Lymphocytes can be collected from circulating blood, bone marrow, cord blood, lymphoid organs or natural secretions including milk and ejaculated semen. The sample can be enriched and purified by means of density gradient centrifugation or other means of separation, including immunomagnetic separation, fluorescence activated cell sorting, column filtration and similar techniques.

In the context of this invention, references to "mononuclear cells" for donor cells should be interpreted as references to lymphocytes, being lymphocytes at more than 95% of the mononuclear cells separated on a Hystopaque gradient. Lymphocytes have been characterised by immunocytochemestry and do not express cytokeratins as well as lamin A/C that are typical of differentiated cells: Galli & al. 1995, Proc. of the Italian Soc. of Vet. Sci. XLIX, 303-304; Rober, RA & al., 1990, J. Cell Sci. 95, 587-598. To this extent, the hematopoietic lineage shares some characteristics with embryonic cells that are also negative for cytokeratins and lamin A/C: Galli et al. 1994, Zygote 2: 385-389. This could explain in part the successful reprogramming of these nuclei into the cytoplasm of enucleated matured oocytes.

Freshly collected lymphocytes can be cultured in vitro and are karyotypically normal. This latter characteristic is a prerequisite for the normal development of any individual, but it is not guaranteed by other cell types that have to be cultured for a length of time and

where a degree of aneuploidy always occurs. Lymphocytes can also be cultured in vitro for a time sufficient to use recombinant DNA technology to alter their genetic constitution: Bordignon & al., 1995, Science 270, 470-475.

In principle this invention is applicable to all animals, but it will be useful in particular for livestock species such as cattle, buffaloes, sheep, goat, pigs, horses, rabbit and other species of economic relevance. It can also be used to preserve genetic material or to generate animals of endangered, exotic or rare species. In humans, it could find beneficial application in its use to generate embryonic stem cells from a patient as a source of compatible undifferentiated cells to be used in transplantation for the therapy of degenerative diseases.

After the reconstruction procedure whereby a lymphocyte or the nucleus of a lymphocyte is reprogrammed into the cytoplasm of an enucleated oocyte, there are several options for which this invention could be used. Lymphocytes can be easily cryobanked and therefore offer an economic way of storing germplasm of animals. When the embryo is reconstructed it can be used not for reproduction but to generate undifferentiated embryonic cell lines to be used in cell therapy of the individual that donated them thus overcoming the problem of rejection. If the embryos obtained are used for the generation of an animal this can be done directly by transferring the pre-implantation embryos to a final recipient that will carry the embryo to term, or the embryo can be subjected to serial nucleus transfer and therefore generate further embryos in a process that is more efficient and probably will increase the chances of reprogramming the cell nucleus because is exposed to the egg's cytoplasm more than once in a short period.

The steps involved in the cloning of an animal using this invention are summarised:

Step 1 - isolate the donor cell required from circulating blood or other tissue; enrichment for the fraction of cells that is more efficient in the procedure; optionally the cells can be genetically

modified during a period of in vitro culture using recombinant DNA technology.

At this stage the cells can be cultured, cryopreserved following one of the established protocols for later use or used immediately for nucleus transfer.

Step 2 - maturation of the oocytes harvested from donor females at slaughter or from live donors and removal of the egg's metaphase plate to prepare the so called 'recipient cytoplast'.

Step 3 - transfer of the nucleus obtained in step 1 by direct micro-injection of the cell or of the isolated nucleus directly in the cytoplasm of the enucleated oocyte or by other means such as cell fusion that can be achieved using intact donor cells with chemical, electrical or viral means. Microinjection is preferred and, preferably, the cell is transferred with the cell membrane broken. Established cell fusion methods include the use of fusion-promoting chemicals, such as polyethylene glycol; the use of a virus such as the Sendai virus; and electrical stimulation.

After introduction of the lymphocyte, the oocyte is activated to mimic sperm entry and start the developmental programme of the oocyte. The delay if microinjection is used to introduce the lymphocyte is typically 2-6 hours before activation. Cold shock as well as aging can activate the cytoplast. Activation may also be by inducing calcium oscillations in the embryo by chemical (ionophore) or physical (electric current) means, following which the embryo is exposed to kinases and protein synthesis inhibitors that facilitate the exit from the metaphase arrest that is maintained upon new protein synthesis. Typical chemical activation would be by 6-dimethylamino purine or cycloheximide. This exposure would be subsequent to the ionophore and the exposure is typically for several hours (e.g., 4-6 hours).

Step 4 - develop the reconstructed embryo to a stage where it can be transferred to the uterus of the final recipient or subject to a serial cloning procedure by disgregating the embryos obtained in single cells

and restarting from step 2. Various known systems of culturing embryos can be used successfully.

The steps involved in the preparation of a stem cell line using this invention are summarised:

Obtain a preimplantation stage (morula or blastocyst, preferably a blastocyst) embryo following steps 1-4 described in the previous example.

Step 5 - Remove the zona pellucida of the embryo. Optionally, the inner cell mass may be isolated from the embryo, for example by mechanical means or by immunosurgery. The intact embryo or the isolated inner cell mass is plated and cultured. Various known systems of culturing embryonic stem cells may be used. The culture takes place on a monolayer of fibroblasts and/or in defined media supplemented with the necessary growth factors (leukaemia inhibitor factor, stem cell factor and others), which are required to maintain the embryonic cell in an undifferentiated state.

Step 6 - Subculture using, for example, mechanical or enzyme dispersal of the embryonic cell outgrowths in new culture vessels to expand the number of cells until a stable cell line is obtained.

Step 7 - The cell line may be frozen for long term storage or the genetic constitution of the cells genetically modified using recombinant DNA technology.

Step 8 - Following genetic modification, the embryonic cell may be used in the cloning of a mammal by following steps 2 to 4.

Recloning procedures can also be carried out by developing the embryo of steps 1-4 to the fetus stage in vivo and sampling cells from the fetus for use in the preparation of further embryos.

EXAMPLE

This is an example of the use of the invention for the cloning of a cattle but similarly it can be applied to other species.

Step 1 - Cell isolation

A blood sample was taken from a cow of proven genetic value by venipuncture with heparinized vacutainer. The blood was diluted 1:1 with phosphate buffer saline (PBS) and 7 ml were layered on 3 ml of a density gradient (Hystopaque density 1083 g/cm3, Sigma) and centrifuged at 1500g for 15-30 minutes, the mononuclear cells stopping at the plasma Hystopaque interface. The 0.5 - 1 ml band of mononuclear cells (lymphocytes) was recovered, transferred into a new centrifuge tube, further diluted with PBS and centrifuged again to wash the cells. This step was repeated once and the cells were finally resuspended in an appropriate culture medium.

Lymphocytes were cryopreserved in medium supplemented with 10-20% serum and 10% DMSO (dimethyl sulfoxide) and packed for example in plastic straws (normally used to pack bovine semen), each containing convenient working aliquots of cells (0.5-2 million cells) required in each day the method of the invention was carried out.

Step 2 - Preparation of cytoplasts

Oocytes at the second metaphase were used. These oocytes were collected from ovaries of slaughtered animals or by ultrasound guided transvaginal recovery from live donors. After collection immature oocytes were subjected to a 15-20 hour maturation period until they reached the second metaphase, following protocols described by Galli & Lazzari, Anim. Reprod. Sci. 42, 371-379, 1996. Oocytes at the end of the maturation period were denuded from the surrounding follicle cells and treated with a fluorescent dye (Hoechst 33342) that stains the chromosomes in the metaphase plate. With the aid of a micromanipulator under an inverted microscope using a micropipette, the first polar body, with a small volume of cytoplasm surrounding it, was removed and checked under fluorescent light for the presence of the metaphase plate. After enucleation, the cytoplasts obtained in this way were returned to culture.

Step 3 - Embryo reconstruction

Lymphocytes prepared in step 1 and cytoplasts prepared in step 2 were transferred to a manipulation chamber under an inverted microscope and each cytoplast was injected with a small micropipette with one cell as described in Tesarik & Mendoza, Human Reproduction 11, 772-779; 1996. It was important to make sure that the cytoplast membrane was broken and the cell or its nucleus was effectively injected in the cytoplasm ready to undergo the reprogramming events necessary to support embryonic development. Failure to break the cytoplast membrane adequately could leave the lymphocyte deposited in a "pocket" of the oocyte membrane. After injection the cytoplasts were returned to culture for a period generally of 2-4 hours.

About 70-80% of the cytoplasts survived the injection procedure. At this stage the oocytes were activated by exposing sequentially the reconstructed embryos (cytoplasts) for 5-7 minutes to 5 μM of Ionomycin (Sigma) and then to 2.5 mM 6-DMAP (Dimethyl amino purine, Sigma) for 4-5 hours: Susko-Parrish & al. 1994, Dev. Biol. 166, 729-739. This mimics sperm entry and will start the developmental programme of the oocyte.

Step 4 - Embryo development

Following activation, the reconstructed embryos were transferred to an in vitro culture system generally used to develop fertilised oocytes to blastocysts. Embryos were cultured in microdrops of SOF (synthetic oviductal fluid, Gardner & al. 1994; Biol. Reprod, 50, 390-400) in an atmosphere of 5% CO_2, 5%O_2 in nitrogen at 38.5 °C.

A proportion of the embryos (5%) developed to the blastocyst stage and could therefore be transferred to synchronised recipients or frozen for subsequent transfer.

With such embryos a pregnancy rate of over 50% (58%) was achieved, the most advanced stage obtained was a pregnancy aborted at 195 days of gestation (a normal bull calf of about 10 kg). The results are shown in Table 1. Most of the pregnancies resulted in abortions between 60-

120 days. Two pregnancies developed to 180 days from 31 transfers. The first is the 195 day mentioned above, the second an oversized bull calf of 19kg which had to be aborted.

Second Generation Cloning

In the first generation cloning only 5% of the reconstructed embryos developed to the blastocyst stage. By this, 16 cell stage by day 4 and compacted morula by day 6 are presumably those embryos in which the reprogramming of the introduced nucleus has occurred.

To increase the efficiency of the procedure the first generation products are subjected to a second generation cloning. This second generation cloning is more efficient because it uses blastomeres (16, 32, 64 cells stages, preferably 32 or over cells stage) and also gives the DNA a second chance for reprogramming because it is recycled back into the cytoplast.

Embryos obtained in the first generation cloning were exposed to calcium and magnesium free HBSS (Hanks balanced salt solution) for 2-4 hours to separate the embryo into single isolated blastomeres.

Cytoplasts were prepared as described in step 2 and first activated (as described in step 3) before the blastomere nucleus was transferred. In this case, the intact blastomere was transferred to the perivitelline space of the cytoplast and electrofused. The fusion rate was usually high (in excess of 80%). Reconstructed embryos at this stage were transferred to the culture system described above. The results are shown in Table 1. 19 such recloned embryos were transferred and 10 pregnancies were established, a pregnancy rate of over 50% (53%).

A successful birth has been achieved; a live and healthy calf originating from an embryo of the second generation cloning. The original lymphocyte was collected from a bull.

TABLE 1

Embryo development following lymphocyte injection or recloning.

	No. replicates	No. injected	No. survived %	No. cleaved %	No. developed %
Direct injection (development to blastocyst)	25	1923	1377 71.61	1059 76.91	71 5.16
Direct injection (development to morula 16-64 cells)	7	540	371 38.70	296 79.78	36 9.70
Recloned from morula (development to blastocyst)	7	462*	412 89.18	321 77.91	66 16.02

* "No. injected" is "No. fused" because recloning from blastomeres requires cell fusion not direct injection

Pregnancies	Totals	Cloning	Recloning
No. transfers	50	31	19
No. pregnancies	28	18	10
Pregnancy rate (%)	56.00	58.06	52.63
Developed to 90 days	10	5	5
Developed to 180 days	3	2	1
Developed to term	1	0	1

EFFECT OF THE INVENTION

The present invention provides a source of donor cells for nuclear transfer techniques which gives advantages over known donors. The use of lymphocytes makes for very easy sample collection, which can be from adult animals of known characteristics. The supply of donor cells is not limited. The donor cells can be readily modified in vitro using recombinant DNA technology.

Claims

1. A method of reconstructing a mammalian embryo, the method comprising transferring a lymphocyte into a suitable recipient.

2. The method according to claim 1 further comprising the step of isolating the nucleus of the lymphocyte before transfer of said nucleus into the recipient.

3. Method according to claim 1 or 2 in which the mammal is an ungulate species.

4. Method according to any preceding claim further comprising the step of genetically modifying the nucleus of the lymphocyte.

5. Method according to any preceding claim in which the recipient is an enucleate oocyte.

6. A method of reconstructing a mammalian embryo comprising reconstructing a first generation embryo by the steps of a method according to any of claims 1 to 5 and further comprising transferring a cell from the said first generation embryo to a suitable recipient to form a second generation embryo.

7. A method of reconstructing a mammalian embryo comprising reconstructing a first generation fetus by development of a first generation embryo reconstructed by a method of any of claims 1 to 5, preparing fetal fibroblast cultures therefrom and transferring cells from the said fetal fibroblast cultures to a suitable recipient to form a second generation embryo.

8. A method according to claim 7 further comprising the step of genetic modification of the cells of the fetal fibroblast cultures prior to second generation cloning.

9. A method of preparing a mammal, the method comprising:

reconstructing a mammalian embryo using a method according to any preceding claim;

allowing the embryo so produced to develop to term; and optionally breeding from the mammal so formed.

10. A method of preparing embryonic stem cell lines, comprising reconstructing a mammalian embryo using a method according to any of claims 1 to 8 and transferring the embryo to a culture system.

11. A method of preparing embryonic stem cell lines, comprising reconstructing a mammalian embryo using a method according to any of claims 1 to 8; isolating the inner cell mass of the embryo from the embryo and transferring the inner cell mass to a culture system.

12. A method according to claim 10 or 11 further comprising the step of genetic modification of the stem cells.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02624

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01K67/027 C12N5/06 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 98 07841 A (UNIV MASSACHUSETTS) 26 February 1998 (1998-02-26) * page 1, line 4-18, page 8, line 15-18, Table 1 *	1,2,5,6, 10-12 3,4,7-9

Y	SCHNIEKE A ET AL: "Human Factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts" SCIENCE, vol. 278, 19 December 1997 (1997-12-19), pages 2130-2133, XP002067036 abstract	3,4,7-9

P,X	WO 98 30683 A (UNIV MASSACHUSETTS A PUBLIC IN) 16 July 1998 (1998-07-16) page 23, line 17	1-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
20 September 1999	05/10/1999

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 07668 A (CAMPBELL KEITH HENRY STOCKMAN ;ROSLIN INST EDINBURGH (GB); WILMUT) 6 March 1997 (1997-03-06) page 8, line 13-19	
A	RITCHIE W A ET AL: "INTRACYTOPLASMIC NUCLEAR INJECTION AS AN ALTERNATIVE TO CELL FUSION FOR THE PRODUCTION OF BOVINE EMBRYOS BY NUCLEAR TRANSFER" JOURNAL OF REPRODUCTION AND FERTILITY. SUPPLEMENT, vol. 5, 1 January 1995 (1995-01-01), page 60 XP000607293	
A	DU PASQUIER L ET AL: "Transplantation of nuclei from lymphocytes of adult frogs into enucleated eggs: special focus on technical parameters" DIFFERENTIATION, vol. 8, no. 1, 1977, pages 9-19, XP002115398 abstract	
A	WO 97 07669 A (ROSLIN INST EDINBURGH ;CAMPBELL KEITH HENRY STOCKMAN (GB); WILMUT) 6 March 1997 (1997-03-06)	
P,A	KATO Y ET AL: "Eight calves cloned from somatic cells of a single adult" SCIENCE, vol. 282, no. 5396, 11 December 1998 (1998-12-11), pages 2095-2098, XP002115305	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/02624

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1, 2 and 4-12 (all partially) have not been searched in so far the embryo is a human embryo, as this subject matter falls within the exeptions to patentability of Article 53 (a) EPC.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

.ormation on patent family members

Interr ›nal Application No
PCT/EP 99/02624

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9807841 A	26-02-1998	AU 4044397 A	06-03-1998
		EP 0934403 A	11-08-1999
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		CA 2229657 A	06-03-1997
		CN 1202085 A	16-12-1998
		CZ 9800604 A	15-07-1998
		EP 0847237 A	17-06-1998
		GB 2318792 A	06-05-1998
		HU 9802485 A	01-02-1999
		NO 980846 A	29-04-1998
		PL 325336 A	20-07-1998
WO 9707669 A	06-03-1997	AU 6831096 A	19-03-1997
		CA 2229568 A	06-03-1997
		CN 1202084 A	16-12-1998
		CZ 9800608 A	15-07-1998
		EP 0849990 A	01-07-1998
		EP 0930009 A	21-07-1999
		GB 2318578 A	29-04-1998
		GB 2331751 A	02-06-1999
		HU 9900234 A	28-05-1999
		NO 980845 A	29-04-1998
		PL 325331 A	20-07-1998

PATENT COOPERATION TREA

PCT

SN

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
HRW/39471		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 02624	19/04/1999	20/04/1998
Applicant		
LTR C.I.Z DI ASSOCIAZIONE ITALIANA ALLEVATORI		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/02624

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1, 2 and 4-12 (all partially) have not been searched in so far the embryo is a human embryo, as this subject matter falls within the exeptions to patentability of Article 53 (a) EPC.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02624

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01K67/027 C12N5/06 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 98 07841 A (UNIV MASSACHUSETTS) 26 February 1998 (1998-02-26) * page 1, line 4-18, page 8, line 15-18, Table 1 *	1,2,5,6, 10-12 3,4,7-9

Y	SCHNIEKE A ET AL: "Human Factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts" SCIENCE, vol. 278, 19 December 1997 (1997-12-19), pages 2130-2133, XP002067036 abstract	3,4,7-9

P,X	WO 98 30683 A (UNIV MASSACHUSETTS A PUBLIC IN) 16 July 1998 (1998-07-16) page 23, line 17	1-12

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 1999	05/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 07668 A (CAMPBELL KEITH HENRY STOCKMAN ;ROSLIN INST EDINBURGH (GB); WILMUT) 6 March 1997 (1997-03-06) page 8, line 13-19	
A	RITCHIE W A ET AL: "INTRACYTOPLASMIC NUCLEAR INJECTION AS AN ALTERNATIVE TO CELL FUSION FOR THE PRODUCTION OF BOVINE EMBRYOS BY NUCLEAR TRANSFER" JOURNAL OF REPRODUCTION AND FERTILITY. SUPPLEMENT, vol. 5, 1 January 1995 (1995-01-01), page 60 XP000607293	
A	DU PASQUIER L ET AL: "Transplantation of nuclei from lymphocytes of adult frogs into enucleated eggs: special focus on technical parameters" DIFFERENTIATION, vol. 8, no. 1, 1977, pages 9-19, XP002115398 abstract	
A	WO 97 07669 A (ROSLIN INST EDINBURGH ;CAMPBELL KEITH HENRY STOCKMAN (GB); WILMUT) 6 March 1997 (1997-03-06)	
P,A	KATO Y ET AL: "Eight calves cloned from somatic cells of a single adult" SCIENCE, vol. 282, no. 5396, 11 December 1998 (1998-12-11), pages 2095-2098, XP002115305	

3

INTERNATIONAL SEARCH REPORT

[redacted]ormation on patent family members

International Application No
PCT/EP 99/02624

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9807841 A	26-02-1998	AU 4044397 A	06-03-1998
		EP 0934403 A	11-08-1999
WO 9830683 A	16-07-1998	AU 6014598 A	03-08-1998
WO 9707668 A	06-03-1997	AU 6830996 A	19-03-1997
		CA 2229657 A	06-03-1997
		CN 1202085 A	16-12-1998
		CZ 9800604 A	15-07-1998
		EP 0847237 A	17-06-1998
		GB 2318792 A	06-05-1998
		HU 9802485 A	01-02-1999
		NO 980846 A	29-04-1998
		PL 325336 A	20-07-1998
WO 9707669 A	06-03-1997	AU 6831096 A	19-03-1997
		CA 2229568 A	06-03-1997
		CN 1202084 A	16-12-1998
		CZ 9800608 A	15-07-1998
		EP 0849990 A	01-07-1998
		EP 0930009 A	21-07-1999
		GB 2318578 A	29-04-1998
		GB 2331751 A	02-06-1999
		HU 9900234 A	28-05-1999
		NO 980845 A	29-04-1998
		PL 325331 A	20-07-1998

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 December 1999 (06.12.99)	
International application No. PCT/EP99/02624	**Applicant's or agent's file reference** HRW/39471
International filing date (day/month/year) 19 April 1999 (19.04.99)	**Priority date** (day/month/year) 20 April 1998 (20.04.98)
Applicant GALLI, Cesare et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   19 November 1999 (19.11.99)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. Cupello
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

5000

Translation

09673939

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 436J PCT 375	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FR99/00963	International filing date (*day/month/year*) 22 April 1999 (22.04.99)	Priority date (*day/month/year*) 24 April 1998 (24.04.98)
International Patent Classification (IPC) or national classification and IPC A47C 23/06		
Applicant DELAHOUSSE ET FILS		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22 November 1999 (22.11.99)	27 April 2000 (27.04.2000)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FR99/00963

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

☐ the international application as originally filed.

☒ the description, pages 1-20, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-18, filed with the letter of 13 April 2000 (13.04.2000),
Nos. _____, filed with the letter of _____.

☒ the drawings, sheets/fig 1/10-10/10, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-18	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	1-18	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	1-18	**YES**
	Claims		**NO**

2. Citations and explanations

1) Independent Claim 1

Closest prior art: FR-A-2 738 471 (D1) discloses, cf. Figures 1 to 6, a device 3 acting as an end piece to support the end of a lath 2a, 2b, according to the preamble of the independent claim.

Problem: To make an end piece in the form of a clip which will attach the end of a lath efficiently to the long section of a bedstead.

Solution: The device claimed consists of a clip comprising a sill that can be fixed in its central part by intermediate means to a bedstead frame. There are hook-shaped turn ups on the sill ends. The said hooks serve to surround the sides of a lath and extend slightly over its top by 2 to 3mm. The clip is made of high-density polyethylene-like material with high elastic memory.

In the embodiment according to Figure 6 of D1, the seating 30 of device 3 is not intended to be fixed in its central part to the bedstead frame (long section 5).

EP-A-0 637 427 discloses a device which forms the

end piece for supporting the end of a lath 13. The device is rectangular, and comprises an opening 14' which cooperates with a groove 15 in the lath, cf. Figure 4.

DE-U-297 13 359 discloses a device acting as an end piece for supporting the end of a lath 12, 13, comprising two bent back parts 15 having protruding members on their inner surfaces, cf. Figures 5 and 6. Said parts are arranged on the long section 18 of a bedstead.

Consequently, the subject matter of independent Claim 1 meets the requirements set out in PCT Article 33(1).

Dependent Claims 2 to 18

The dependent claims specify advantageous embodiments of the device which is the subject matter of the independent claim, and also meet the requirements set out in PCT Article 33(1).

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

1) Description

1.1) Contrary to PCT Rule 5.1(a)(ii), the description does not indicate the relevant prior art set out in document D1 and does not cite that document.

1.2) Under the terms of PCT Rule 11.13(l) reference signs not mentioned in the description should not appear on the drawings, and vice versa. This requirement is not met for reference sign 66, cf. page 16, line 21 and for sign 74, cf. page 18, line 13.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* HRW/39471

Box No. I TITLE OF INVENTION

Source of Nuclei for Nuclear Transfer

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LTR C.I.Z Di Associazione Italiana Allevatori
Via Porcellasco 7-f,
26100 Cremona,
ITALY.

A company incorporated under the laws of Italy.

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: ITALY

State *(that is, country)* of residence: ITALY

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Dr. Cesare Galli
Via Persico 191/G,
26100 Cremona,
ITALY.

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: ITALY

State *(that is, country)* of residence: ITALY

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WAKERLEY, Helen Rachael,
Reddie & Grose,
16 Theobalds Road,
London. WC1X 8PL.
UNITED KINGDOM.

Telephone No. +44 171 242 0901

Facsimile No. +44 171 242 3290/0286

Teleprinter No. 25445

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Dr. Giovanna Lazzari,
Via Persico 191/G,
26100 Cremona,
ITALY.

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: ITALY

State *(that is, country)* of residence: ITALY

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AL** Albania
- ☒ **AM** Armenia
- ☒ **AT** Austria
- ☒ **AU** Australia
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria
- ☒ **BR** Brazil
- ☒ **BY** Belarus
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China
- ☒ **CU** Cuba
- ☒ **CZ** Czech Republic
- ☒ **DE** Germany
- ☒ **DK** Denmark
- ☒ **EE** Estonia
- ☒ **ES** Spain
- ☒ **FI** Finland
- ☒ **GB** United Kingdom
- ☒ **GD** Grenada
- ☒ **GE** Georgia
- ☒ **GH** Ghana
- ☒ **GM** Gambia
- ☒ **HR** Croatia
- ☒ **HU** Hungary
- ☒ **ID** Indonesia
- ☒ **IL** Israel
- ☒ **IN** India
- ☒ **IS** Iceland
- ☒ **JP** Japan
- ☒ **KE** Kenya
- ☒ **KG** Kyrgyzstan
- ☒ **KP** Democratic People's Republic of Korea
- ☒ **KR** Republic of Korea
- ☒ **KZ** Kazakhstan
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova
- ☒ **MG** Madagascar
- ☒ **MK** The former Yugoslav Republic of Macedonia
- ☒ **MN** Mongolia
- ☒ **MW** Malawi
- ☒ **MX** Mexico
- ☒ **NO** Norway
- ☒ **NZ** New Zealand
- ☒ **PL** Poland
- ☒ **PT** Portugal
- ☒ **RO** Romania
- ☒ **RU** Russian Federation
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia
- ☒ **SK** Slovakia
- ☒ **SL** Sierra Leone
- ☒ **TJ** Tajikistan
- ☒ **TM** Turkmenistan
- ☒ **TR** Turkey
- ☒ **TT** Trinidad and Tobago
- ☒ **UA** Ukraine
- ☒ **UG** Uganda
- ☒ **US** United States of America
- ☒ **UZ** Uzbekistan
- ☒ **VN** Viet Nam
- ☒ **YU** Yugoslavia
- ☒ **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ AE United Arab Emirates
- ☒ ZA South Africa
- ☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 20 April 1998	9808325.6	United Kingdom		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date (day/month/year)	Number	Country (or regional Office)
04.02.99	RS 102174 GB	EPO

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	12
claims	:	2
abstract	:	1
drawings	:	–
sequence listing part of description	:	–
Total number of sheets	:	19

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: EPO Search Report.

Figure of the drawings which should accompany the abstract: –

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

WAKERLEY, Helen Rachael
Agent of the Applicant

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
HRW/39471		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/02624	19/04/1999	20/04/1998
International Patent Classification (IPC) or national classification and IPC A01K67/027		
Applicant CONSORZIO INCREMENTO ZOOTECNICO S.R.L. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/11/1999	18.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Stolz, B Telephone No. +49 89 2399 8416

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/02624

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

   Description, pages:

   1-13 as originally filed

   Claims, No.:

   1-13 as originally filed

2. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 4, 7-10, 13
	No:	Claims	1, 2, 5, 6, 11, 12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Priority

Priority documents have not been at the Examiner's disposition at the time of establishing this repport. It has been established under the assumption of valid priority rights.

2. Reasoned statement

2.1. The application describes the cloning of embryos from mononuclear cells. i.e. from lymphocytes. The procedure resulted in the successful cloning of a calf.

2.2. Novelty (Art. 33(2) PCT)

WO98/07841 (D1) describes the establishment of transspecies - ES like cells. The so produced cells can be used as nuclear donors for nuclear transplantation (p. 8, lines 15-18). The method consists of transferring the nucleus of an adult, i.e. differentiated human cell into an enucleated animal oocyte. Suitable donor cells are listed on p. 12 of D1, amongst these are lymphocytes and mononuclear cells (lines 9 and 10). Preferred recipient oocytes are obtained from ungulates, most preferably bovine (p. 12, line 25). As explained on p. 4, lines 2 to 4, of the instant application, the term "embryo" includes morulas of between 8 and 32 cells, and blastocysts of 64 cells or more. Table 1 of D1 describes the use of lymphocytes as donor cells developing to an early morula stage. Thus, D1 anticipates the subject matter of claims 1, 2, 5, 6, 11 and 12.

2.3. Inventive step (Art. 33(3) PCT)

In light of the general statements in the introduction of D1 (p. 2, lines 13-15; p. 4, lines 13-15) and Schnieke et al., the subject matter of claims 3, 4, 7 to 9, 10 and 13 represent obvious modifications of the procedure described in D1.

WO97/07669 (D2) describes a method of reconstituting an animal embryo which involves the transfer of a donor nucleus to a suitable recipient cell. The method is said not to be restricted to particular donor cells and includes partially and fully differentiated cells. The only difference between the instant application and D2

lies in the use of lymphocytes as donor cells. Such cells are not specifically mentioned in D2, and the question in assessing inventive step is, if they represented an obvious alternative to the person of skill. Lymphocytes and mononuclear cells present a selection from a larger list of possible donor cells known in the art (e.g. D1, lines 6 to 16). Such a selection can only be inventive when associated with an unexpected effect. The presently claimed method does not appear to provide an unexpected effect in comparison with the method of D2. Therefore, also when using D2 as the closest item of prior art and combining its teaching with the general knowledge of the person of skill, claims 1 to 13 lack inventive step.

3. Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO98/30683	16.07.1998	05.01.1998	10.01.1997

4. Certain observations

4.1. According to p. 4, 3rd paragraph, the term "mononuclear cells" is used synonymously with the term "lymphocytes". The difference between the scope of claims 1 and 2 is therefore unclear.

RLe

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EUROPEAN PATENT OFFICE

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 39471/HRW

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/EP99/02624	19 April 1999 (19.04.99)	20 April 1998 (20.04.98)

Title of invention: Source of Nuclei for Nuclear Transfer

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Consorzio Incremento Zootechnico S.R.L.
Via Porcellasco 7-f,
26100 Cremona,
ITALY.

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Dr. Cesare Galli
Via Persico 191/G,
26100 Cremona,
ITALY.

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Dr. Giovanna Lazzari,
Via Persico 191/G,
26100 Cremona,
ITALY.

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/EP99/02624

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Helen Rachael Wakerley,
Reddie & Grose,
16 Theobalds Road,
London, WC1X 8PL.
UNITED KINGDOM.

Telephone No.: +44 20 7242 0901

Facsimile No.: +44 20 7242 3290/0286

Teleprinter No.: 25445

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[x] the international application as originally filed

the description [x] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[x] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/EP99/02624

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:	2	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter	:	1	sheets	☐	☐
6. other *(specify)*	:		sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

WAKERLEY, Helen Rachael
Applicant's Representative

November 19th 1999.

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5. below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 December 1999 (06.12.99)	
International application No. PCT/EP99/02624	**Applicant's or agent's file reference** HRW/39471
International filing date (day/month/year) 19 April 1999 (19.04.99)	**Priority date** (day/month/year) 20 April 1998 (20.04.98)
Applicant GALLI, Cesare et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   19 November 1999 (19.11.99)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

C. Cupello

Telephone No.: (41-22) 338.83.38